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# Development of the Epidermal Cells of the Pinnules of *Adiantum raddianum* C.Presl (Pteridaceae): Environmental Adaptive Plastidial Characteristics

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Abstract.—Plants have different strategies for adapting to environmental conditions, such as characteristics that allow them to be more efficient in shady or sunny environments. Those that grow in dimly lit environments often have a photosynthetic epidermis. However, *Adiantum raddianum*, known as the maidenhair fern, is a species found in sunny environments that has this characteristic. Within the abaxial and adaxial epidermal cells of the leaf pinnules of this species, there are arm-like projections where chloroplasts agglomerate. The goal of this study was to describe the development of the epidermal cells of *A. raddianum*, describe the morphological characteristics of the chloroplasts in these cells, and interpret any cytological characteristics that this species might have as a result of an adaptive survival strategy. The study found that the arm-like projections within the epidermis develop when the leaves are young and still exhibit circinate vernation. Cytological observations revealed a plastidial dimorphism, where there was variation in the arrangement of the thylakoid system, and the presence of stromules, which may help establish a connection among chloroplasts and between these organelles and mitochondria and peroxisomes. Descriptions of the stromules and plastidial dimorphism, made in this study, can be included with other known epidermal adaptive strategies (e.g., plastidial movement and mucilage secretion), which help this plant to survive under different environmental conditions.

Key Words.—*Adiantum*, chloroplasts, dimorphism, stromules, adaptation

Within a natural environment, light varies in duration, amount, and spectral quality. Many plants have developed adaptations in response to these variations, for example, within their leaves (Vogelmann and Martin, 1993). Leaves that grow predominantly in the shade often vary in thickness and size and have variable stomata and vein density when compared to those that grow in the sun. In addition, the leaf mesophyll, which is usually the main photosynthetic stratum, may vary in the number of palisade and spongy parenchyma layers and in the compression of the cells that form the tissue (Evans, 1999; Dickinson, 2000).

According to Esau (1965), the functions of the aerial epidermis are transpiration restriction, protection, gas exchange via stomata, and the storage of water and metabolic products. However, Esau also stated that in some cases the epidermis might assume additional functions that could cause it to have

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atypical characteristics, such as the ability to photosynthesize or secrete metabolic products. The presence of chloroplasts in the leaf epidermis is common in pteridophytes (mainly in filmy ferns), aquatic plants, and some terrestrial gymnosperms and angiosperms, especially those that grow in the shade (Esau, 1965; Mickel, 1972; Dickinson, 2000).

In members of the leptosporangiate *Adiantum*, the leaf pinnules have an epidermis that acts as a photosynthetic stratum, in addition to participating in gas exchange and the transportation of metabolites (through its veins). The importance of the epidermal cells in this genus is greater in some species, especially when the mesophyll of the species contains sparsely distributed arm-like cells that form projections on the internal periclinal walls where chloroplasts agglomerate (Wylie, 1948). This characteristic is found in the abaxial and adaxial epidermal cells of *Adiantum raddianum* C. Presl (popularly known as the maidenhair fern), which is a common species in South and Central America, Mexico and the Old World (Wylie, 1948; Ogura, 1972; Davidse *et al.*, 1995; Gómez and Arbeláez, 2009). Although a photosynthetic epidermis is generally associated with shade plants, *A. raddianum* grows in semi-shady to sunny environments, such as on cliffs along roadsides, on the banks of watercourses (Sehnem, 1972), and along the edges of forests (Senna and Kasmirczak, 1997).

Silva *et al.* (2007) observed the accumulation of vacuolar mucilage and the motion of chloroplasts within the epidermis of *A. raddianum*. The motion of the chloroplasts was observed on the external periclinal walls of immature leaves, and was restricted to projections corresponding to the internal periclinal walls when the leaves were mature. These characteristics were interpreted as environmental adaptations.

The goal of this study was to describe the development of the epidermal cells of *A. raddianum*, describe the morphological characteristics of the chloroplasts in these cells, and interpret any cytological characteristics that *A. raddianum* might have as a result of an adaptive survival strategy.

## Material and Methods

*Collection site and stages of development.*—The material was collected at Morro Santana, in Porto Alegre, Rio Grande do Sul, Brazil (30°02′00″S to 30°04′40″S and 51°06′30′W to 51°09′00″W). The plants were collected on a cliff with exposed soil, which received six to eight hours of sunlight per day. For this study, four developmental stages were identified based on morphological and anatomical differences. Pinnules of all stages were always collected at the same time. Three stages of pinnule development were collected from leaves with circinate vernation and the fourth stage collected was of leaves that were fully expanded.

*Optical microscopy.*—Median portions of sterile pinnules were prepared using a fixative of 1% glutaraldehyde and 4% formaldehyde, in a 0.1 M sodium phosphate buffer, at pH 7.2 (McDowell and Trump, 1976). They were then dehydrated in a graded ethanol series and placed in an ethanol-

chloroform graded series (Purvis *et al.*, 1964). The material was then embedded in hydroxyethylmetacrylate resin (Gerrits and Smid, 1983). Transverse and longitudinal sections (2 μm thick) were cut using a rotary microtome (Microm HM 340) with a glass knife. The sections were stained with Toluidine Blue O (C.I. 52040), at pH 4.4 (Feder and O'Brien, 1958). In addition, a histochemical test using Lugol's iodine (Sass, 1951) was performed in order to detect starch grains. The sections were observed using a bright field Leica DM R microscope.

*Transmission electron microscopy (TEM).*—The median region of sterile pinnules was fixed in a solution of 2.5% glutaraldehyde and 2% paraformaldehyde in a 0.1 M sodium phosphate buffer, at pH 7.2 (Roland and Vian, 1991). The material was postfixed in a solution of 2% osmium tetroxide ($OsO_4$) and 0.8% potassium ferricyanide ($K_3Fe(CN)_6$) in a 0.2 M sodium phosphate buffer, at pH 7.2 (Weber, 1992). Subsequently, the material was dehydrated in an acetone series and embedded in low-viscosity epoxy resin (Spurr, 1969). Transverse, ultrathin sections were obtained using a Leica Ultracut UCT ultramicrotome with a diamond knife. The sections were adhered to copper grids and stained with a 2% aqueous uranyl acetate (Bozzola and Russel, 1999) and 5% lead citrate solution (Hanaichi *et al.*, 1986). The analyses were performed using a Jeol 1200 ExII transmission electron microscope.

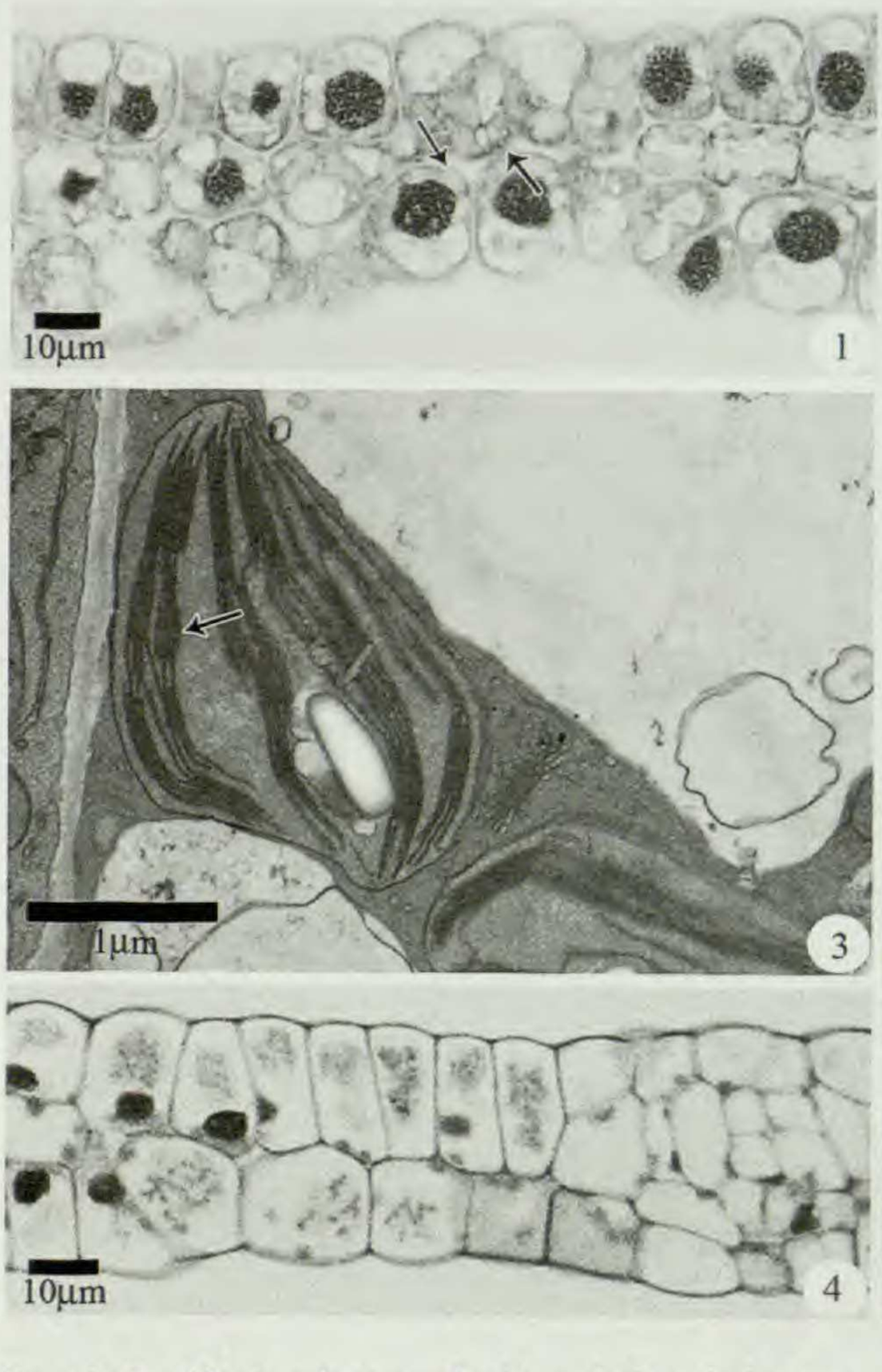
*Confocal laser scanning microscopy.*—In order to analyze the morphology of the chloroplasts in the epidermis, epidermal peels were made using fully expanded pinnules. The fresh material, in paradermal view, was incubated with Calcofluor (Pacini *et al.*, 1999) and mounted in immersion oil. The slides and coverslips were sealed with nail polish. The material was analyzed using an Olympus FV1000 confocal microscope using an ×60 oil immersion objective. Excitation filters were used to analyze the Calcofluor test (at band width 330–385 nm) and chlorophyll autofluorescence (at band width 530–550 nm). Z-series profiles of 20 optical sections were collected at 1μm using the Olympus FV1000 software.

## Results

The most undifferentiated pinnules were represented by those of "stage one", where all epidermal cells were rectangular and lacked large intercellular spaces (Fig. 1). In this stage, contact among epidermal cells was observed (Fig. 1, arrows). These cells contained dense cytoplasm and a large vacuole, and many mitochondria were observed near the chloroplasts (Fig. 2, arrows). A smooth endoplasmic reticulum was observed next to the other organelles. The epidermal chloroplasts had starch granules (Fig. 3, asterisk), thylakoids, and were starting to form grana (Fig. 3, arrow).

In the second stage, the epidermal cells were more anticlinally elongated and there were no intercellular spaces (Fig. 4). Another relevant characteristic observed was the formation of protrusions of the plastidial membrane, which were free of thylakoids (Fig. 5, asterisks).

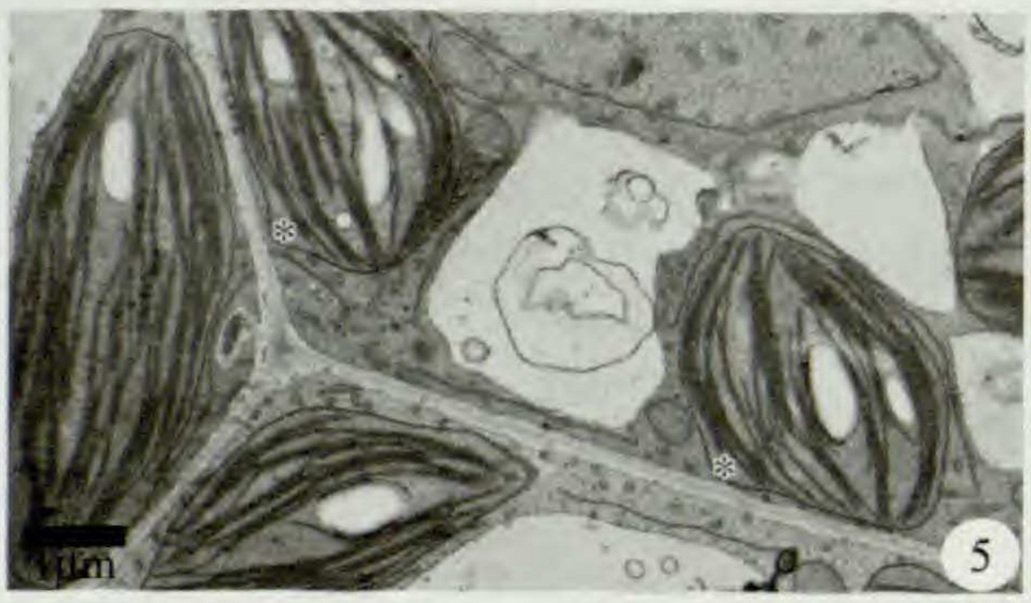
The third and final developmental stage collected while the leaves had circinate vernation, showed the initial development of the arm-like projections

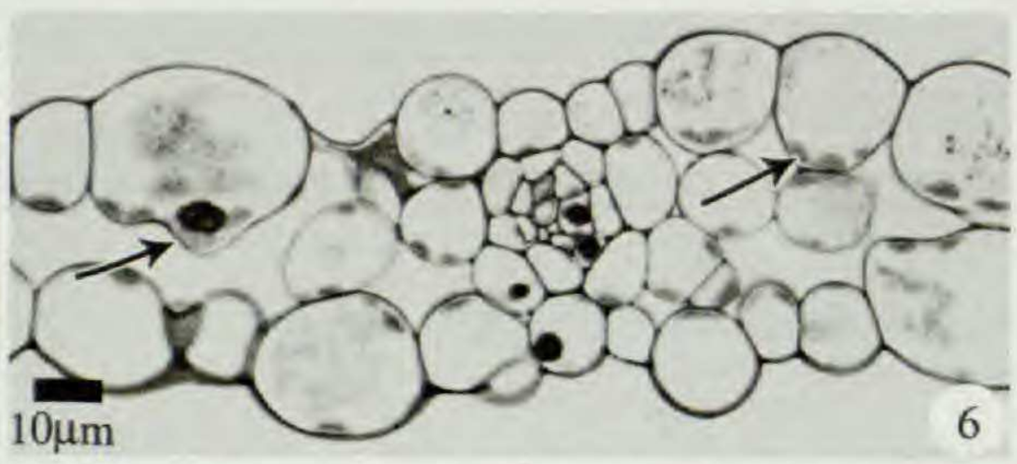


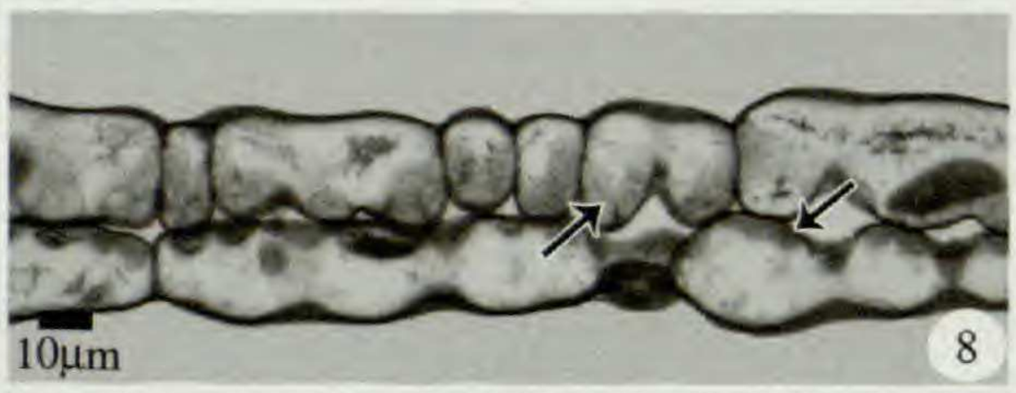

FIGS. 1–4. Photomicrographs (1 and 4) and electron micrographs (2 and 3) of transverse sections of pinnules of *Adiantum raddianum*. **1)** Stage one, general aspect of the epidermal cells and contact between the surfaces of the epidermis (arrows). **2)** Stage one, montage of two electron micrographs of the general aspect of the cell with large vacuole (v) and the association of chloroplasts and mitochondria (arrows). **3)** Stage one, chloroplasts with thylakoids and initial formation of grana. **4)** Stage two, elongated epidermal cells without intercellular spaces.

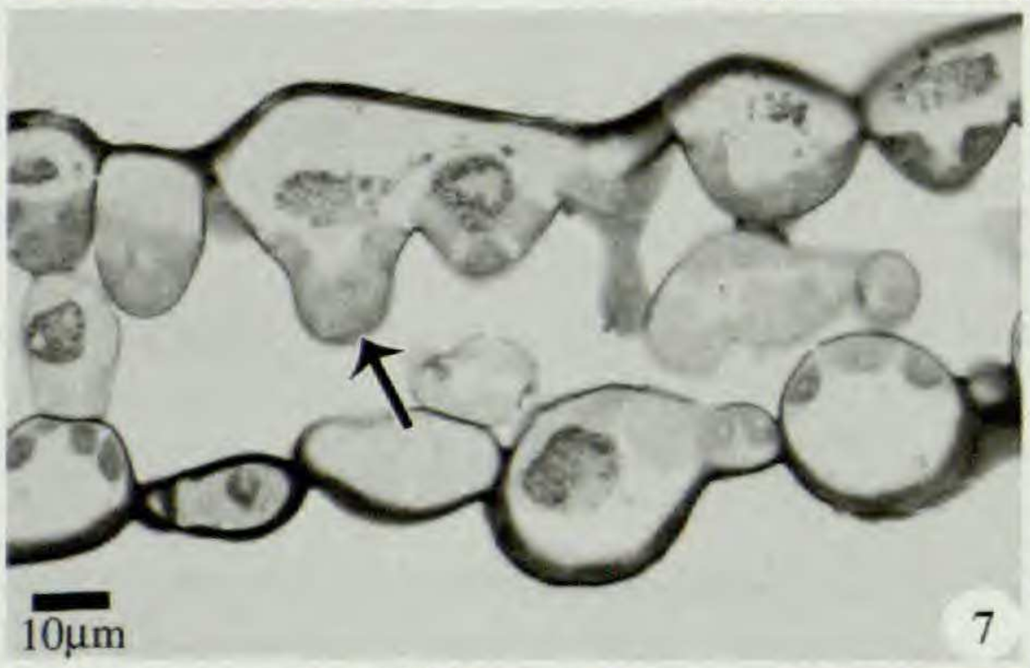
(in both epidermal surfaces) where the chloroplasts gather (Fig. 6, arrows). Few mesophyll cells were present, which resulted in wide intercellular spaces during leaf expansion.

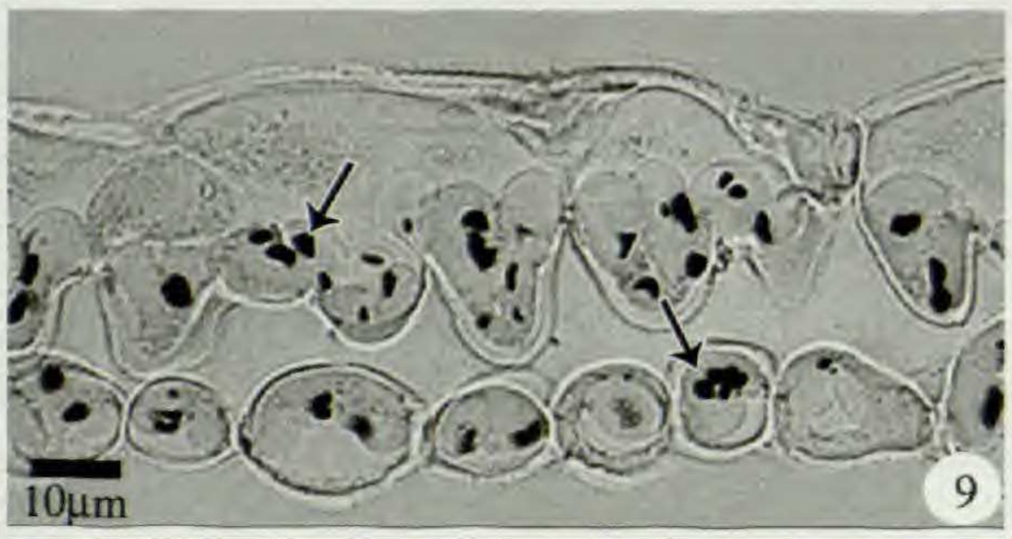
In the fourth stage, which was of pinnules that were completely expanded, the arm-like projections within the adaxial and abaxial epidermal cells were fully developed, establishing the region where the chloroplasts gather (Fig. 7). In longitudinal section, the arm-like projections of the adaxial surface were more prominent than those of the abaxial surface (Fig. 8). The starch granules were visible in the chloroplasts of both epidermal surfaces (Fig. 9, arrows), and were not noticeably different in size or quantity when compared to those observed in the initial stages. However, the analysis of transversal TEM sections revealed a plastidial dimorphism related to the orientation of the thylakoids and the grana system (Fig. 10). Chloroplasts showed two types of membrane organization, one with the thylakoids oriented in all directions

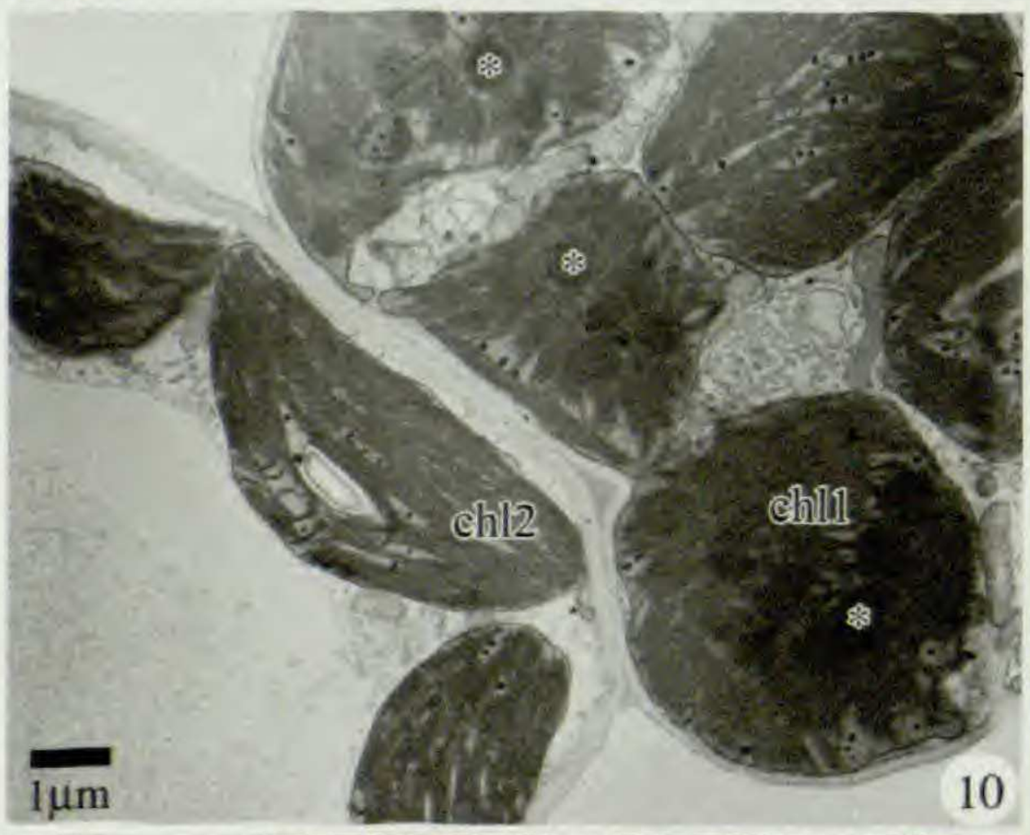


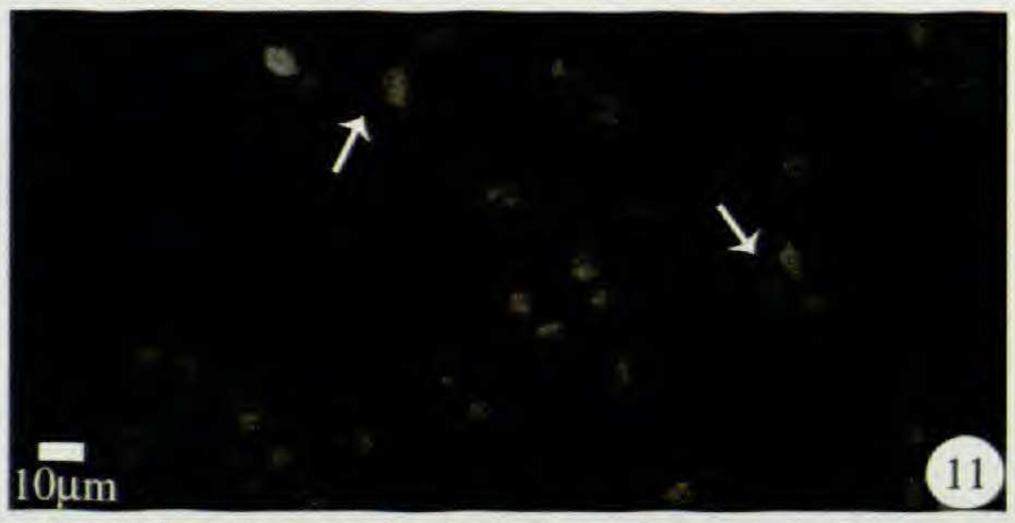



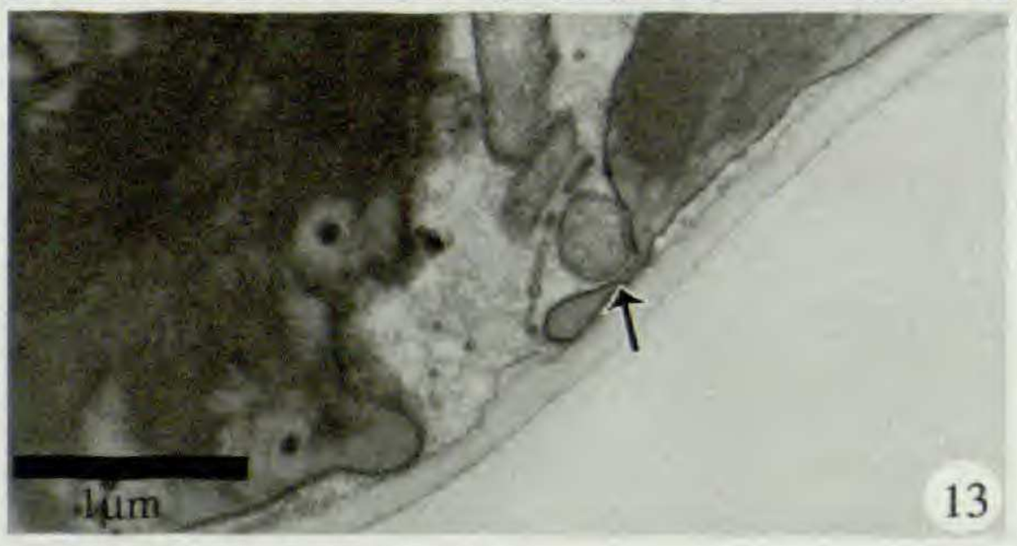






FIGS. 5–9. Photomicrographs (6, 7, 8, 9, 11) and electron micrographs (5 and 10, 12, 13) of transversal and longitudinal sections of pinnules of *A. raddianum*. **5)** Stage two, initial development of the membrane projections free from thylakoids (asterisks). **6)** Stage three, development of the arm-like projections (arrows) in adaxial epidermal cells and the presence of intercellular spaces. **7)** Stage four, general aspect of the epidermal cells with chloroplasts accumulated in the arm-like projections. **8)** Stage four, longitudinal section, elongated epidermal cells and arm-like projections in both surfaces (arrows). **9)** Stage four, detection of starch granules in the chloroplasts of adaxial and abaxial epidermal cells (arrows). **10)** Stage four. Clear dimorphism between chloroplasts, type Chl1 and Chl2, grana in frontal view (asterisks). **11)** Stage four, discoid-shaped chloroplasts in the cells of the adaxial surface of the epidermis (arrows). **12)** Stage four, contact between chloroplasts through the stromules. **13)** Stage four, contact between the projection of the plastidial membrane and mitochondria (arrow).

(Fig. 10, Chl1) and the other with this system of membranes oriented in only one direction (Fig. 10, Chl2). Both types were observed in the same leaf surface (data not shown).This type of dimorphism was based on the internal structure of the chloroplasts, but was not reflected in the external morphology; analysis of the plastidial morphology using confocal microscopy showed a morphological resemblance between the chloroplasts, all of which were discoid to slightly elliptic (Fig. 11).

In both types, the protrusion of the plastidial membrane, called a stromule, was evident. When the leaves were mature (the forth stage), the stromules were more elongated, allowing them to contact neighboring chloroplasts (Fig. 12, arrow), or mitochondria (Fig. 13, arrow) and peroxisomes (not shown).

## Discussion

Light assimilation by leaves depends on their anatomical structure, and is associated with the number and distribution of chloroplasts per cell and the amount of chlorophyll pigment (Vogelmann and Martin, 1993). The development of arm-like projections within the adaxial and abaxial epidermal cells begins when the leaves are immature and still exhibit circinate vernation. Wylie (1948) stated that besides the chloroplasts being more efficiently located to properly capture light (resembling palisade parenchyma), such cells could facilitate the transport of metabolites among the veins.

In an anatomical study with species of the Pteridaceae, including some species of *Adiantum*, Graçano *et al.* (2001) observed the presence of chlorophyllous epidermal cells with arm-like projections in certain leaf blades. This characteristic was observed in plants that grew in shady environments. However, differently from the species described by Graçano *et al.* (2001), *A. raddianum* is a species that grows in sunny environments and has arm-like projections that orient the chloroplasts in a way that will not damage the photosynthetic apparatus when capturing light.

Besides the quick development of the arm-like projections as an adaptative characteristic to help direct light capture, Silva *et al.* (2007) observed the accumulation of vacuolar mucilage and the movement of chloroplasts in *A. raddianum*, which, when the pinnules were immature, were positioned on the external periclinal walls and, when mature, were restricted to the projections corresponding to the internal periclinal walls. These characteristics were interpreted as environmental adaptations.

The structure of the chloroplasts and the relative amounts of plastidial components are clear expressions of adaptation to environmental conditions (Bonatti and Fornasiero, 1990). Depending on the environmental conditions, the chloroplasts can be morphologically round or flat (Esau, 1965). The polymorphism of chloroplasts has been reported for some plants, and in some cases more than seven forms have been found inside a single leaf (Fisher and Evert, 1982). Besides this, there are reports about differentiated zones in relation to the organization of plastidial membranes inside a single chloroplast, where there is polarization among thylakoids with and without

the formation of grana, as described by Machado *et al.* (1986), and in bizonoplast, as described by Sheue *et al.* (2007). All of these characteristics denote the extreme flexibility of these organelles to different environmental conditions, and the chloroplast characteristics described in leaf blade of *A. raddianum* also reveals this environmental plasticity.

*A. raddianum* has a leaf blade composed mainly of epidermis and manifests structural variations among the chloroplasts. Similarly, in *Teratophyllum rotundifoliatum* (Bonap.) Holttum, a pteridophyte that grows in extremely shady environments, there is a dimorphism among the chloroplasts within the adaxial and abaxial epidermal cells, which is also the predominant leaf tissue in this species (Nasrulhaq-Boyce and Duckett, 1991). Jagels (1970) also verified a dimorphism among the chloroplasts occurring in both faces of the epidermis in the microphylls in five species of *Selaginella*, in which mesophyll is practically absent. However unlike previous studies, our study found that both types of chloroplasts occur within the same leaf surface.

The orientation of the grana lamellae in all directions in pteridophytes (such as in *Selaginella* [Jagels, 1970] and *T. rotundifoliatum* [Nasrulhaq-Boyce and Duckett, 1991]), Cycadaceae (Bonatti and Fornasiero, 1990), and angiosperms that grow in the shade (Anderson *et al.,* 1973) could be associated with an increase in the absorption of weak and diffuse solar radiation that reaches the forest floor (Nasrulhaq-Boyce and Duckett, 1991). *Adiantum raddianum*, however, occurs in sunny environments, and has the same characteristic of chloroplasts in the epidermis and plastidial dimorphism of the thylakoids' arrangement as the species analyzed by the aforementioned authors.

In plants with C4 metabolism, the dimorphism of the chloroplasts is related to the size of the chloroplast, the architecture of the thylakoid, and the starch content. This dimorphism can be observed between the bundle-sheath and the mesophyll cells and is associated with different biochemical functions. Nasrulhaq-Boyce and Duckett (1991) associated the plastidial dimorphism, in the pteridophyte *T. rotundifoliatum*, to the presence of metabolic differences, which resembled a plant with a C4 metabolism. Therefore, it is possible that in *A. raddianum*, each plastidial type could have a particular metabolic specificity.

Besides photosynthesis, chloroplasts are necessary for the biosynthesis of starch, amino acids, fatty acids, (Neuhaus and Emes, 2000), carotenes, purines, and pyrimidines (Köhler *et al.,* 1997). For this reason, it is presumed that the physical interaction between chloroplasts and the other organelles would turn the transference of metabolites among cellular compartments into a more efficient process (Kwok and Hanson, 2004). The external surface of the chloroplasts has the ability to form tubules filled with stroma, called stromules (Köhler and Hanson, 2000). Similar structures were observed in the chloroplasts of *A. raddianum*, from the youngest stage to the oldest, in both types of chloroplasts. Stromules have the ability to exchange metabolites with other organelles (Köhler and Hanson, 2000), with the cytoplasm (Natesan *et al.*, 2005), and even with the nucleus (Kwok and Hanson, 2004). Such structures have been observed before in species exposed to saline and high

temperatures (Holzinger *et al.*, 2007) and plants from alpine environments, which are naturally exposed to intense solar radiation (Lütz and Engel, 2007), and they could be considered an additional adaptation developed by plants to face certain climate conditions. Although, *A. raddianum* is a plant that is structurally similar to plants that live in shade, the occurrence of stromules indicates a possible adaptation to environments with high amounts of light. This is the first time that these structures are described for a species with a phenotype that is similar to a shade plant (with a photosynthetic epidermis), but grows in a sunny environment.

In conclusion, the plastidial characteristics observed during the development of the epidermal cells of the pinnules of *A. raddianum* seem to be associated with the adaptive ability of this species. The discrimination of the arm-like projections in immature pinnules, the presence of both forms of chloroplasts, and the presence of stromules in the epidermal chloroplasts can be added to characteristics (plastidial movement and the secretion of mucilage) already described by Silva *et al.* (2007) for the epidermis of *A. raddianum*, forming a set of adaptive strategies at several structural levels that this species uses to cope with particular environmental conditions.

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## Literature Cited

Anderson, J. M., D. J. Goodchild and N. K. Boardman. 1973. Composition of photosystems and chloroplast structure in extreme shade plants. Bioch. Biophys. Acta 325(3):573–585.

Bonatti, P. M. and R. B. Fornasiero. 1990. Developmental pattern and structural organization of leaf chloroplasts in *Lepidozamia peroffskyana.* Aust. J. Bot. 38(1):53–62.

Bozzola, J. J. and L. D. Russel. 1998. *Electron microscopy. Principles and Techniques for Biologists.* Jones and Bartlett Publishers, Boston.

Davidse, G. S., S. Sousa, S. M. Knapp and S. M., (eds.) 1995. *Flora Mesoamericana. Volumen 1. Psilotaceae a Salviniaceae.* Universidad Nacional Autónoma de México, México. D.F.

Dyckinson, W. C. 2000. Integrative Plant Anatomy: Harcourt, San Diego.

Esau, K. 1965. *Plant anatomy. 2nd ed.* John Wiley & Sons Inc., New York.

Evans, J. R. 1999. Leaf anatomy enables more equal access to light and $CO_2$ between chloroplasts. New Phytol. 143:93–104.

Feder, N. and T. P. O'Brien. 1958. Plant microtechnique. Some principles and new methods. Amer. J. Bot. 55:123–142.

Fisher, D. G. and R. F. Evert. 1982. Studies on the leaf of *Amaranthus retroflexus* (Amaranthaceae): chloroplast polymorphism. Bot. Gaz. 143(2):146–155.

Gerrits, P. O. and L. Smid. 1983. A new, less polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. J. Microsc. 132:81–85.

GÓMEZ, L. D. and A. L. ARBELÁEZ. 2009. *Flora de Nicaragua. Helechos (Tomo IV).* Stevens, W. D., O. M. Montiel and A. Pool, eds. Monographs in systematic botany from the Missouri Botanical Garden, volume 116. Missouri Botanical Garden Press, St. Louis.

GRAÇANO, D. A., A. A. AZEVEDO and J. PRADO. 2001. Anatomia foliar das espécies de Pteridaceae do Parque Estadual do Rio Doce (PERD)-MG. Rev. Bras. Bot 24:337–347.

HANAICHI, T., T. SATO, T. IWAMOTO, J. MALAVASI-YAMASHIRO, M. HOSHIRO and N. MIZUNO. 1986. A stable lead by modification of Sato's method. J. Elect. Microsc. 35:304–306.

HOLZINGER, A., O. BUCHNER, C. LÜTZ and M. R. HANSON. 2007. Temperature-sensitive formation of chloroplast protrusions and stromules in mesophyll cells of *Arabidopsis thaliana*. Protoplasma 230:23–30.

JAGELS, R. 1970. Photosynthetic apparatus in *Selaginella*. 2. Changes in plastid ultrastructure and pigment content under different light and temperature regimes. Can. J. Bot. 48(10): 1853–1870.

KÖHLER, R. H., J. CAO, R. Z. WARREN, W. W. WEBB and M. R. HANSON. 1997. Exchange of protein molecules through connections between higher plant plastids. Science 276:2039–2042.

KÖHLER, R. H. and M. R. HANSON. 2000. Plastids tubules of higher plants are tissue-specific and developmentally regulated. J. Cell Sci. 113:81–89.

KWOK, E. Y. and M. R. HANSON. 2004. Plastids and stromules interact with the nucleus and cell membrane in vascular plants. Plant Cell Rep. 23:188–195.

LÜTZ, C. and L. ENGEL. 2007. Changes in chloroplast ultrastructure in some high-alpine plants: adaptation to metabolic demands and climate? Protoplasma 230(3–4):183–192.

MACHADO, R. D., W. R. SANT'ANNA and M. BENCHIMOL. 1986. Internal dimorphism of chloroplasts in the leaves of *Peperomia pellucida* (L.) H.B.K. Protoplasma 134(2–3):73–77.

MCDOWELL, E. M. and B. R. TRUMP. 1976. Histological fixatives for diagnostic light and electron microscopy. Arch. Pathol. Lab. Med. 1000:405–414.

MICKEL, J. T. 1972. A "Film Fern" in the genus *Cystopteris*. Am. Fern. J. 62(3):93–95.

NASRULHAQ-BOYCE, A. and J. G. DUCKETT. 1991. Dimorphic epidermal cell chloroplasts in the mesophyll-less leaves of an extreme-shade tropical fern, *Teratophyllum rotundifoliatum* (R. Bonap.) Holtt.: a light and electron microscope study. New Phytol. 119:433–444.

NATESAN, S. K. A., J. A. SULLIVAN and J. C. GRAY. 2005. Stromules: a characteristic cell-specific feature of plastid morphology. J. Exp. Bot. 56(413):787–797.

NEUHAUS, H. E. and M. J. EMES. 2000. Nonphotosynthetic metabolism in plastids. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:111–140.

OGURA, Y. 1972. *Comparative anatomy of vegetative organs of the pteridophytes*. Gebrüger Borntraeger, Berlin.

PACINI, E., G. G. FRANCHI and M. RIPACCIOLI. 1999. Ripe pollen and histochemistry of some gymnosperms. Plant Syst. Evol. 217:81–99.

PURVIS, M. J., COLLIER, D. C. and WALLS, D.. 1964. *Laboratory techniques in botany*. Butterworths, London.

ROLAND, J. C. and B. VIAN. 1991. General preparation and staining of thin sections. *In*: Hall, J. L. and E. Hawes, eds. *Electron Microscopy of Plant Cells*. Academic Press, London.

SASS, J. E. 1951. *Botanical microtechinique*. Iowa State University Press, Ames.

SEHNEM, A. S. J. 1972. Pteridáceas. *In:* Reitz, R. ed. *Flora Ilustrada Catarinense*. Tipografia e Livraria Blumenauense; Itajaí.

SENNA, R. M. and C. KASMIRCKAZ. 1997. Pteridófitas de um remanescente florestal no Morro da Extrema, Porto Alegre, RS. Rev. Fac. Zoo. Vet. Agro. 4(1):33–48.

SHEUE, C.-H., V. SARAFIS, R. KIEW, H.-Y. LIU, A. SALINO, L.-L. KUO-HANG, Y.-P. YANG, C.-C. TSAI, C.-H. LIN, J. W. H. YOUNG and M. S. B. KU. 2007. Bizonoplast, a unique chloroplast in the epidermal cells of microphylls in the shade plant *Selaginella erythropus* (Selaginellaceae). Am. J. Bot. 94(12):1922–1929.

SILVA, F. S., A. A. MASTROBERTI and J. E. A. MARIATH. 2007. Aspectos anatômico-funcionais das células epidérmicas de pínulas de *Adiantum raddianum* Presl. (Pteridaceae). Rev. Bras. Bio. 5(supl.1):831–833.

SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrast. Res. 26:31–34.

VOGELMANN, T. C. and G. MARTIN. 1993. The functional significance of palisade tissue: penetration of directional versus diffuse light. Plant Cell Environ. 16:65–72.

WEBER, M. 1992. The formation of pollenkitt in *Apium nodiflorum* (Apiaceae). Ann. Bot. 70:573–577.

WYLIE, R. B. 1948. The dominant role of the epidermis in leaves of *Adiantum*. Amer. J. Bot. 35:465–473.